Imaging & Optical Microscopy: Cell Tissue Imaging

957-Pos Board B743

STICCS Reveals Matrix-Dependent Adhesion Slipping and Gripping in Migrating Cells

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Two-color STICCS (spatio-temporal image cross correlation spectroscopy) is a new image analysis method that calculates space-time autocorrelation and cross correlation functions from fluorescence intensity fluctuations. STICCS generates cellular flow and diffusion maps that reveal interactions and co-transport of two distinct molecular species labeled with different fluorophores. Here we use computer simulations to map the capabilities and limitations of STICCS for measurements on complex heterogeneous environments containing micro- and macrostructures. We then use STICCS to analyze the flux of adhesions in migrating cells imaged using total internal reflection fluorescence (TIRF) microscopy. The data reveal a robust, time-dependent interaction between certain integrins and paxillin in retracting regions where adhesions are sliding and disassembling demonstrating that they move as a complex. In these regions, both $\alpha 6\beta 1$- or $\alpha L\beta 2$-integrins, expressed in CHO.B2 cells, co-flux with paxillin; an analogous co-transport was seen for $\alpha 6\beta 1$-integrin and α-actinin in U2OS. This contrasts with the behavior of the $\alpha 5\beta 1$-integrin and paxillin, which do not co-flux. Our results clearly show that integrins can move in complexes with adhesion proteins in retracting regions of the cell.

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Co-Localization of Environmentally Sensitive Fluorescent Probe and Ellipsometry for Cell-Based Studies

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Ellipsometry is a well-established technique for studying the deposition of thin films in solid state materials. Applying ellipsometry to biological systems has significant challenges due to the wide variety of cellular components which contribute to the signal. We utilize a transparent ellipsometry substrate to perform multi-modality measurements in combination with fluorescence microscopy. We labeled HeK293 cells with the membrane probe Laurdan, and present the comparison of this fluorescence signal with the ellipsometry signal resulting from interactions with the substrate surface. In comparisons of previous works, ellipsometry has been shown to provide effective concentration on cell based screening faster than labeling techniques alone. By combining these two methodologies in a multi-modality system we are able to use the specificity of fluorescence labeling to evaluate the specific origins of the ellipsometry signal.

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Fluorescence Imaging and Analysis of Blood Flow in Connexin-36 Mouse Pancreatic Islets

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Pancreatic islets are central in regulating blood glucose levels. Thus, controlling delivery of secreted hormones such as insulin, glucagon, and somatostatin likely depends upon regulating blood flow through each individual islet. The mechanism of control is expected to have one or more components that are localized to the islets, and perhaps to specific cell types within an islet. In wild type mice we have observed localized regulation of blood flow with varying glycemic conditions. Where, under hypoglycemic conditions there is a greater loss of blood flow in islets than in the exocrine pancreas, while for hyperglycemic conditions both islets and exocrine pancreas are well perfused. We hypothesize that this localized regulation of blood flow is related to coordinated electrical activity in islets that couples into vascular endothelial cells to control blood flow. To test this, we use high-speed confocal microscopy to image blood flow in the pancreas of wild type, Cx36(+/-), and Cx36(-/-) (Connexin 36 knockout in beta cells) mice. Data is collected at different z-positions through the islets and surrounding exocrine tissue, for a series of X-Y-Time scans. Vasculature is labeled with a fluorescent rhodamine dextran in the plasma, and a portion of blood cells are labeled by osmotic shock loading with a fluorescent Alexa dye. Results are presented that quantify differences between the three genotypes. Specifically, average blood flow densities and velocities for whole islets and the exocrine pancreas under glycemic conditions from <50 mg/dl (hypoglycemia) to >300 mg/dl (hyperglycemia) are presented. In addition, localized blood flow differences present in single islets, as a function of glycemic conditions, are analyzed. We expect in Cx36(-/-) mice, with islets that lack coordinated electrical activity, much less localized regulation of blood flow under hypoglycemic conditions.

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Multiphoton Ultraviolet Microscopy Reveals Dopamine Dynamics in Live Brain Tissue

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Intracellular dopamine dynamics is a prelude to the sensation of reward and to motor control, and also a key to understanding substance addiction and Parkinson's disease. Dopamine neurotransmission has been investigated extensively, yet direct optical probing of dopamine has not been possible till now. Here we image intracellular dopamine with sub-micron three-dimensional resolution, by harnessing its auto-fluorescence with two-photon ultraviolet excitation (using a femtosecond optical parametric oscillator with output at 540 nm) and non-epifluorescent detection. The technique is established by first imaging dopamine in the dopaminergic cell line MN9D. These cells appear to be bright, and the signal strength reduces upon amphetamine administration (amphetamines are known to be powerful dopamine releasing agents). We then show that individual dopamine vesicles in the substantia nigra region can be imaged inside cultured brain slices. The cell brightness is much less in regions away from the nigra. Our technique can follow the intracellular events preceding dopamine release induced by depolarization and amphetamine exposure in these slices. This provides a unique assay for following any neurophysiological process that affects the intracellular dopamine dynamics.

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Fluorescence Lifetime Imaging Microcopy of Extravasating Cancer Cells in the Mouse Microenvironment

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Cell migration is fundamental for cancer metastasis, wound healing, stem cell differentiation and development. Here we explore the tumor microenvironment of MDA-MB-231 breast cancer cells using the phasor approach to FLIM in order to map the fluorescence lifetime of autofluorescencent intrinsic bio-chemical species in living tissues. We compared aggressive cancer cells with host mouse cells as they extravasated from the blood vessel wall into surrounding tissue. To visualize the tumor cells we engineered MDA-MB-231 breast cancer cells to express paxillin-GFP, a focal adhesion protein involved in cell migration. To image the response of cancer cells and the surrounding tissue during extravasation, tumor cells were injected in the blood stream of nude mice and imaged using 2-photon excitation to excite EGFP at 900 nm and at 760nm to detect autofluorescence in the microenvironment. A skin flap was opened to visualize blood vessels and recognize the position of colonies of the tumor cells. Two-photon imaging showed that after an initial phase in which cells are non-adherent, some cells spread on the internal surface of blood vessels. After four days many cells appeared on the external side of the blood vessel. Preliminary results show that when cells start to adhere to the blood vessel wall they form focal adhesions as determined by their characteristic elongated features observed in the paxillin-GFP channel. Using the phasor analysis method we map the concentration of collagen, flavins, and NADH by their phasor signature. Cells show autofluorescence indicated of the metabolic states of the cancer cells. This approach could identify cancer cells in tissues based on their autofluorescence signature. Furthermore we could detect the response of surrounding tissue at different stages of cancer-cell extravasation. This work was supported by NIH P50 GM076516, P41 RR03155 and CA132971.

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A Cell Motility Assay Based on Image Correlation Spectroscopy

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The potential of specialized cell types to migrate inside an organism is playing a central role in embryonic development, wound healing, inflammation, and tumor metastasis. Investigating the constituents of the cell migration machinery, such as, cell adhesion, cytoskeleton or extra-cellular matrix proteins, and their modulators therefore is fundamental in basic cell biophysics research and drug discovery alike. A number of cellular assays are available for different aspects of migration, e.g., chemotaxis assays for directed motion along a gradient, scratch assays for void repopulation, or porous membrane invasion assays. The grade of bulk motility is determined from the amount of cells that passed from one area or compartment to another between two time points. In a more